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EFFECT OF CERTAIN CULTURE CONDITIONS ON THE VIRULENCE OF THE  
PLAGUE MICROBE

[Following is the translation of an article by Ye. I. Korobkova, G. Kha. Dyushikyan, L. P. Plavlova, and M. V. Zubova, All-Union Scientific-Research Antiplague Institute "Mikrob" (Saratov), published in the Russian-language periodical ZhMEI (Journal of Microbiology, Epidemiology, and Immunobiology), No 6, 1966, page 146. It was submitted on 22 Jan 1965.]

In a previous work (1964) Dyushikyan showed that prolonged storage of genetically homogeneous virulent strains in flasks on a thick layer of agar (25 ml) led to a loss of virulence without a change in remaining features, including immunogenicity. On beveled agar in test tubes and in a dried state the strains preserved their virulence. For the purpose of experimental confirmation of this fact we conducted serial passages of the highly virulent (Dcl equal to 25 microbes) plague microbe strain No 708 from flask to flask with a thick layer of agar (50 ml). Simultaneously the same reseeds were conducted in test tubes with beveled agar. It was preliminarily established that even a brief existence of a virulent strain on a thick layer of agar exerted an influence on its virulence: in the culture a greater number of avirulent cells was revealed than during cultivation on a thin layer of agar in test tubes. After the 5th and 10th passages on a large volume of agar the virulence of strain No 708 was lowered for mice by 20, and for guinea pigs by 125 times in comparison with the virulence of the initial strain.

On the synthetic medium of Jackson and Burrows (1956) with hemin a non-passaged strain formed a large number of pigmented colonies; as a result of passage through a thick layer of agar the number of pigmented colonies on a medium with hemin was reduced by 14 times. In a strain, passaged through beveled agar in test tubes, the number of pigmented colonies was reduced only by 6 times. The rapid lowering of the virulence of the culture was apparently conditioned by the fact that on a large volume of medium conditions were created which were favorable for the more intensive multiplication of the plague microbe with a frequent replacement of the number of generations and this promoted the adaptation of cells to existence outside of a host organism, to the selection of cells which had adapted to growth on artificial nutrient media, and to a loss of virulence.

→ Frequent reseeds of virulent strains of the plague microbe on a thick layer of agar led to a loss of virulence and a weakening of immunogenic properties. Consequently, by means of prolonged storage (cultivation) of a virulent strain of the plague microbe on a large

volume of nutrient medium with infrequent reseedings (no more than once a year) it is possible to obtain a variant with the properties of vaccine strains for use as a live vaccine.